

**WHAT IS CLAIMED:**

1                    1.        An expression plasmid comprising an RNA polymerase I (pol I)  
2       promoter and pol I terminator sequences, which are inserted between an RNA polymerase II  
3       (pol II) promoter and a polyadenylation signal.

1                    2.        The expression plasmid of claim 1 wherein the pol I promoter is  
2       proximal to the polyadenylation signal and the pol I terminator sequence is proximal to the pol  
3       II promoter.

1                    3.        The expression plasmid of claim 1 wherein the pol I promoter is  
2       proximal to the pol II promoter and the pol I terminator sequence is proximal to the  
3       polyadenylation signal.

1                    4.        The expression plasmid of claim 1 wherein the plasmid corresponds to  
2       a plasmid having a map selected from the group consisting of pHW2000, pHW11 and  
3       pHW12.

1                    5.        The expression plasmid of claim 1, further comprising a negative strand  
2       RNA virus viral gene segment inserted between the pol I promoter and the termination signal.

1                   6.     The expression plasmid of claim 5, wherein the negative strand RNA  
2 virus is a member of the *Orthomyxoviridae* virus family.

1                   7.     The expression plasmid of claim 6, wherein the virus is an influenza A  
2 virus.

1                   8.     The expression plasmid of claim 7, wherein the viral gene segment  
2 encodes a gene selected from the group consisting of a viral polymerase complex protein, M  
3 protein, and NS protein; wherein the genes are derived from a strain well adapted to grow in  
4 cell culture or from an attenuated strain, or both.

1                   9.     The expression plasmid of claim 6, wherein the virus is an influenza B  
2 virus.

1                   10.    The expression plasmid of claim 8 wherein the plasmid has a map  
2 selected from the group consisting of pHW241-PB2, pHW242-PB1, pHW243-PA, pHW245-  
3 NP, pHW247-M, and pHW248-NS.

1                   11.    The expression plasmid of claim 8 wherein the plasmid has a map  
2 selected from the group consisting of pHW181-PB2, pHW182-PB1, pHW183-PA, pHW185-  
3 NP, pHW187-M, and pHW188-NS.

2025-12-11 14:28:28

1                   12.     The expression plasmid of claim 7, wherein the viral gene segment  
2     encodes a gene selected from the group consisting of an influenza hemagglutinin (HA) gene  
3     and a neuraminidase (NA) gene.

1                   13.     The expression plasmid of claim 12, wherein the influenza gene is from  
2     a pathogenic influenza virus strain.

1                   14.     The expression plasmid of claim 12, wherein the plasmid has a map  
2     selected from the group consisting of pHW244-HA, pHW246-NA, pHW184-HA, and  
3     pHW186-NA.

1                   15.     A minimum plasmid-based system for the generation of infectious  
2     negative strand RNA viruses from cloned viral cDNA comprising a set of plasmids wherein  
3     each plasmid comprises one autonomous viral genomic segment, and wherein the viral cDNA  
4     corresponding to the autonomous viral genomic segment is inserted between an RNA  
5     polymerase I (pol I) promoter and terminator sequences, thereby resulting in expression of  
6     vRNA, which are in turn inserted between a RNA polymerase II (pol II) promoter and a  
7     polyadenylation signal, thereby resulting in expression of viral mRNA.

1                   16.     The minimum plasmid-based system of claim 15 wherein the pol I  
2     promoter is proximal to the polyadenylation signal and the pol I terminator sequence is  
3     proximal to the pol II promoter.

1                    17.    The minimum plasmid-based system of claim 15 wherein the pol I  
2   promoter is proximal to the pol II promoter and the pol I terminator sequence is proximal to  
3   the polyadenylation signal.

1                    18.    The plasmid-based system of claim 15, wherein the negative strand  
2   RNA virus is a member of the *Orthomyxoviridae* virus family.

1                    19.    The plasmid-based system of claim 18, wherein the virus is an  
2   influenza A virus.

1                    20.    The plasmid-based system of claim 18, wherein the virus is an  
2   influenza B virus.

1                    21.    The plasmid-based system of claim 19, wherein the viral gene segment  
2   encodes a protein selected from the group consisting of a viral polymerase complex protein,  
3   an M protein and an NS protein; wherein said genes are from a strain well adapted to grow in  
4   cell culture or from an attenuated strain, or both.

1                    22.    The plasmid-based system of claim 19, wherein the viral genomic  
2   segments comprise genes which encode a protein selected from the group consisting of

- 1 hemagglutinin and neuraminidase, or both; wherein said genes are from a pathogenic  
2 influenza virus.

1 23. The plasmid-based system of claim 19 wherein said system comprises  
2 one or more plasmids having a map selected from the group consisting of pHW241-PB2,  
3 pHW242-PB1, pHW243 -PA, pHW244-HA, pHW245-NP, pHW246-NA, pHW247-M, and  
4 pHW248-NS.

1 24. The plasmid-based system of claim 19, wherein said system comprises  
2 one or more plasmids having a map selected from the group consisting of pHW181-PB2,  
3 pHW182-PB1, pHW183 -PA, pHW184-HA, pHW185-NP, pHW186-NA, pHW187-M, and  
4 pHW188-NS.

1 25. A host cell comprising the plasmid-based system of claim 15.

1 26. A host cell comprising the plasmid-based system of claim 18.

1 27. A host cell comprising the plasmid-based system of claim 19.

1 28. A host cell comprising the plasmid-based system of claim 22.

1                   29.    A method for producing a negative strand RNA virus virion, which  
2   method comprises culturing the host cell of claim 25 under conditions that permit production  
3   of viral proteins and vRNA or cRNA.

1                   30.    A method for producing an *Orthomyxoviridae* virion, which method  
2   comprises culturing the host cell of claim 26 under conditions that permit production of viral  
3   proteins and vRNA or cRNA.

1                   31.    A method for producing an influenza virion, which method comprises  
2   culturing the host cell of claim 27 under conditions that permit production of viral proteins  
3   and vRNA or cRNA.

1                   32.    A method for producing a pathogenic influenza virion, which method  
2   comprises culturing the host cell of claim 28 under conditions that permit production of viral  
3   proteins and vRNA or cRNA.

1                   33.    A method for preparing a negative strand RNA virus-specific vaccine,  
2   which method comprises purifying a virion produced by the method of claim 29.

1                   34.    The method according to claim 33, which further comprises  
2   inactivating the virion.

1                    35.     The method according to claim 33, wherein the negative strand RNA  
2   virus is an attenuated virus.

1                    36.     A method for vaccinating a subject against a negative strand RNA virus  
2   infection, which method comprises administering a protective dose of a vaccine of claim 33 to  
3   the subject.

1                    37.     A method for vaccinating a subject against a negative strand RNA virus  
2   infection, which method comprises injecting a protective dose of a vaccine of claim 33  
3   intramuscularly in the subject.

1                    38.     A method for vaccinating a subject against a negative strand RNA virus  
2   infection, which method comprises administering a vaccine of claim 33 intranasally to the  
3   subject.

1                    39.     A method for generating an attenuated negative strand RNA virus,  
2   which method comprises:

- 3                    (a)     mutating one or more viral genes in the plasmid-based system of claim  
4                    15; and  
5                    (b)     determining whether infectious RNA viruses produced by the system  
6                    are attenuated.

1                    40.    A composition comprising a negative strand RNA virus virion, wherein  
2    viral internal proteins of the virion are from a virus strain well adapted to grow in culture or  
3    from an attenuated strain, or both and viral antigen proteins, of the virion are from a  
4    pathogenic virus strain.

1                    41.    A composition comprising a negative strand RNA virus virion  
2    produced by the method of claim 29.